REMARKS

Claims 1, 3-4, 6-7, and 10-14 are pending in the present application. Claims 1, 7 and 12-14 have been amended herein to further clarify the claims. No new matter has been added with the amendments . Claims 2, 5 and 8-9 were previously cancelled.

Objections to the claims

Claims 1 and 12 have been objected to for recitation of "hexonuclease". The Examiner questions whether the term contains a typographical error. Claims 1 and 12 have been amended to appropriately recite "exonuclease". Withdrawal of the objection is, therefore respectfully requested.

Rejections under 35 U.S.C.§112, 1st paragraph

The claims have been rejected under 35 U.S.C.§112, 1st paragraph with the assertion that the features "obtained from a patient who smokes" and "correlating the hTERT copy number to the risk of cancer in the patient" are not supported by the specification. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

Support may be found for "obtained from a patient who smokes" at least on page 3, line 22; as well as the Examples, which used patients who smoke. In addition, the Examples further support the feature of "correlating the hTERT copy number to the risk of cancer in the patient". This feature is additionally supported by the disclosure on pages 3, lines 22-24 and on page 4 of the specification, which state respectively, "an increase of plasma DNA of these subjects [i.e. healthy subjects exposed to risk factors, such as smoking – see page 3, lines 19-22] is predictive of increased risk" and "the amplification rate of hTERT is used as an indication of the DNA total amount in the tested sample." Withdrawal of the rejection is, therefore, respectfully requested.

Rejection under 35 U.S.C.§112, 2nd paragraph

The claims have been rejected under 35 U.S.C.§112,2nd paragraph, with the assertion "the risk of cancer in the patient" is unclear. Applicants traverse this rejection and withdrawal thereof is respectfully requested. The claim language is clear with its plain meaning, i.e. that the risk of cancer in a patient is the likelihood or probability that the patient will develop cancer. However, to

render the claims even more clear the term "patient" has been more amended to more generally recite "subject". Withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. §103

Claims 1, 3-4, 7 and 10-14 have been rejected under 35 U.S.C.§103 as being obvious over Sozzi et al., combined with Cech et al., Chang et al. and Gocke et al.

Claim 6 has been rejected under 35 U.S.C.§103 as being obvious over Sozzi et al., combined with Cech et al., Chang et al. and Gocke et al. and in further view of Wick et al., and Lowe et al., as well as the sequence search report listed on the PTO-892 form attached to the office action.

Cech et al. is newly relied on as allegedly teaching a polynucleotide, which encodes the hTERT polypeptide and the use of the polynucleotide in the hybridization and/or amplification of the hTERT genes. The Examiner further asserts that Cech et al. teach the use of the polynucleotide for diagnostics and prognostic applications. The Examiner also asserts that Cech et al. specifically teach the detection of copy number and the correlation of changes to pathological conditions. Thus, Cech et al. is relied on for teaching the copy number of hTERT gene alleles.

Applicants traverse these rejections and withdrawal thereof is respectfully requested. The examiner asserts that Sozzi et al. disclose circulating DNA quantification with plasma DNA using polymerase chain reaction. However, this assessment of the teachings of Sozzi et al. is incorrect and it is from this incorrect assessment of the teachings of Sozzi et al. that the Examiner concludes that Sozzi et al. in combination with Cech et al. (and the additionally cited references) teach the claimed invention.

As previously discussed, Sozzi et al. disclose circulating plasma DNA quantification in 84 lung cancer patients and 43 cancer-free controls using a completely different method for DNA quantification, which is **not** based on the use of polymerase chain reaction (PCR) and which does **not** use any amplification procedures. The authors in Sozzi et al. used a commercially available kit to quantify the DNA: i.e. the Dip-Stick TM Kit (Invitrogen), which consists of a colorimetric assay using strips upon which a few microliters of DNA, extracted from plasma and **not**

amplified by PCR, are spotted (the method is described in the Material & Methods section of Sozzi et al.).

Sozzi et al. further disclose that PCR was used for the analysis of microsatellite alterations in a selected series of plasma samples. However, this analysis is qualitative and not quantitative and was employed only to demonstrate the presence of tumor-associated genetic changes in plasma circulating DNA, and not for determining the total amount of circulating DNA. Thus, the use of PCR in Sozzi et al. was not indicative or suggestive of measuring the total amount of circulating DNA using PCR.

Cech et al., on the other hand, teach that polynucleotides encoding hTRT or fragments thereof are used "as sense or antisense probes or primers for hybridization and/or amplification of naturally occurring hTRT genes or RNAs" (column 15, lines 23-25). Thus, the goal in Cech et al was the determination of the presence of human-telomerase reverse transcriptase genes or RNA transcripts in a sample. In contrast, according to the method of the instant invention, a particular fragment of hTERT gene (see claim 1) is used for determining the total amount of circulating DNA, by interpolating a calibration curve, which is constructed with known (i.e. pre-determined) amounts of total DNA (of which the hTERT gene represents only a fraction), wherein said interpolation is effected starting from the hTERT DNA copy number measured in the test sample by means of quantitative PCR amplification of the hTERT fragment.

In other words, the present invention is based on the finding that the amount of amplified hTERT gene fragment - once interpolated in an appropriate calibration curve obtained with known amounts of DNA (including hTERT fragment as well as many other different sequences) - correlates with the total amount of circulating DNA, which in turn correlates with risk of cancer in the tested subject. This ability to correlate the total amount of circulating DNA based on the amount of amplified hTERT gene fragment (interpolated with a calibration curve) to the risk of cancer was first identified by the inventors, and Cech et al. is completely silent about this. Indeed, while Cech et al. teach how to isolate or amplify hTRT genes or RNAs from a sample they are silent about using hTRT for determining circulating total DNA in a plasma sample, and

Sozzi et al. similarly fail to teach or suggest such missing features, since they do not disclose DNA quantification based on polymerase chain reaction (PCR).

The concept underlying the invention may be more elucidated by a consideration of Chang et al. (page 6 of the office action). In discussing Chang et al., the Examiner acknowledges that "quantification of a sample containing an unknown number of target sequences typically is carried out with reference to a "standard curve" generated from a series of amplifications of samples containing a target sequence in a range of known amounts". This assertion, however, does not apply to the instant invention, wherein the standard curve is not generated from a series of amplifications of samples containing known amounts of the target sequence (in this case, hTERT), but rather from samples containing known amounts of total DNA (of which hTERT represents only a fraction). In fact, not all circulating DNA molecules that can be found in a plasma sample correlate univocally with the total DNA present in the sample itself, as much of it is degraded or otherwise unsuitable to that purpose.

The hTERT fragment amplified with the method of invention, instead, was found to correlate with the total DNA amount, and this finding was unexpected. Yet more unexpected was the finding by the inventors that the total amount of circulating DNA correlates with the risk of cancer in smokers. The total amount of circulating DNA in plasma includes both tumor-derived and host-derived DNA and therefore its measurement by the method of invention allows to determine the effects of an interaction of the tumor with its microenvironment. Thus, the instant method takes a completely different path than known methods of diagnosing or prognosing cancer, which are rely on the detection of specific tumor markers or indicators, such as mutations and mRNA overexpression of cancer-related genes. A fundamental aspect of the present invention is that it does not measure a tumor-specific genetic alteration. The method of instant invention cannot be achieved by the reference teachings. Nor is there any suggestions or motivation to further modify the reference teachings to achieve the instant invention.

As such, claims 1, 3-4, 7 and 10-14 are not obvious over Sozzi et al., combined with Cech et al., Chang et al. and Gocke et al., nor is claim obvious over Sozzi et al., combined with Cech et al., Chang et al. and Gocke et al. and in further view of Wick et al., and Lowe et al., as well as the

sequence search report listed on the PTO-892 form attached to the office action. Withdrawal of the rejections is therefore respectfully requested.

In view of the above amendments and remarks, Applicant believes the pending application is in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact MaryAnne Armstrong, Ph.D., Reg. No. 40,069, at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

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Respectfully submitted,

By _____

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